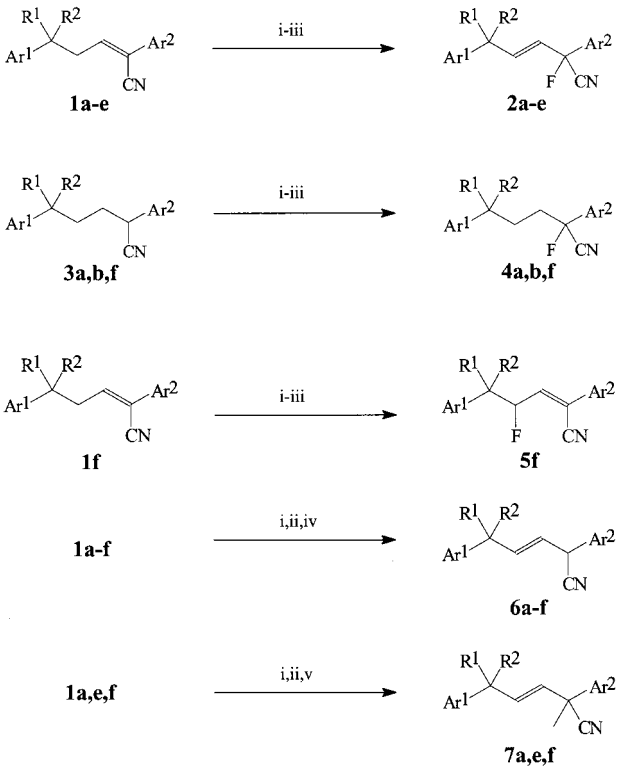

Figure 1(a). The regioselectivity of electrophilic fluorination compared with methylation and acidic quench of the anion. (i) LiHMDS, −78°C to 0°C, 1 h; (ii) −78°C; (iii) $(PhSO_2)_2NF$; (iv) 2M HCl; (v) excess MeI.

3a,b,f, to yield the expected products **4a,b,f**. Yields for the reaction varied from 40 to 90% and no diastereoselectivity was observed for the products **2a–d**. Regioselective control of fluorination *alpha* to the cyano group was complete except in the reaction of **1f**. In this case fluorination yielded **5f,** the opposite regioisomer to that expected and although the yield was moderate (33%) this was the only regioisomer detected. Varying reaction conditions did not produce the desired regioisomer. To examine whether this change in regioselectivity was determined before addition of the electrophile, the anion of **1f** was quenched with 2 M hydrochloric acid and iodomethane. The products, **6f** and **7f** respectively, showed the same regioselectivity observed for **6a–e, 7a** and **7e,** so it is only the reaction of **1f** with the fluorinating agent that generates unexpected regiochemistry. The difference in reactivity of the anion of **1f** compared with **1a–e** is unlikely to be based upon steric grounds, as **1a–d** are less hindered and **1e** more hindered, nor is it due to hyperconjugation. The explanation may involve delivery of the fluorine by an interaction of the cyclopropane ring with the fluorinating reagent but this hypothesis has not been tested.

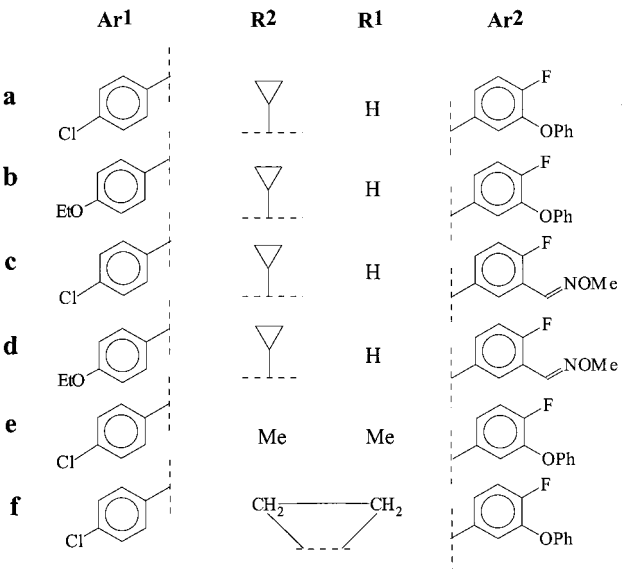

Figure 1(b). Substituents in the analogues depicted in Fig. 1(a).

Results of bioassay of these compounds will be reported elsewhere.

ACKNOWLEDGEMENTS

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Resistance to glyphosate in *Lolium rigidum*

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Abstract: Annual ryegrass (*Lolium rigidum*) is a widespread and important weed of Australia and populations of this weed have developed resistance to most major herbicides, including glyphosate. The possible mechanisms of resistance have been examined in one glyphosate-resistant *Lolium* population. No major differences were observed between resistant and susceptible biotypes in respect of (i) the target enzyme (EPSP synthase), (ii) DAHP synthase, the first enzyme of the target (shikimate) pathway, (iii) absorption of glyphosate, or (iv) translocation. Following treatment with glyphosate, there was greater accumulation of shikimate (derived from shikimate-3-Pi) in susceptible than in resistant plants. In addition, the resistant population exhibited cross-resistance to 2-hydroxy-3-(1,2,4-triazol-1-yl)propyl phosphonate, a herbicide which, although structurally similar to glyphosate, acts at an unrelated target site. On the basis of these observations we speculate that movement of glypho-

sate to its site of action in the plastid is involved in the resistance mechanism.

Keywords: glyphosate; resistance mechanism; *Lolium rigidum*; ryegrass

Following 15 years of successful use (corresponding to a total of *c*40 treatments), glyphosate failed to control a population of the widespread grass weed, *Lolium rigidum* Gaud growing in an orchard near Orange, NSW, Australia. Resistance was confirmed in pot dose-response experiments and survivors (of treatment at 450 g AE ha^{-1} in pots) were further inter-crossed to yield a population which consistently exhibited 7 to 11-fold resistance when compared to the survival rates of a standard susceptible population.[1] Here we describe a study to elucidate the mechanism of resistance.

Glyphosate acts by inhibiting 5-enolpyruvyl shikimate-3 phosphate (EPSP) synthase, a plastidic enzyme involved in the biosynthesis of chorismate,[2] a common intermediate required for the biosynthesis of aromatic amino acids as well as auxin, quinones, and other essential components of plants. We investigated whether resistant plants were altered, either in EPSP synthase, or in the first enzyme in the chorismate pathway, 3-deoxy-D-arabinoheptulosonic acid-7-phosphate (DAHP) synthase. The latter enzyme, also located within the plastid, is thought to control flux through the pathway via feedback regulation by arogenate.[2] Stem and leaf material from two-, three- and five-leaf plants was frozen in liquid nitrogen, mixed with polyvinylpyrrolidone, ground to a powder and resuspended into K^+ Hepes buffer (90 mM; pH 7.5) containing $(NH_4)_2MoO_4$, (0.2 mM) KF (9 mM), EDTA (1 mM), dithiothreitol (DTT; 5 mM), ascorbate (10 mM), 4-(2-aminoethyl)benzenesulfonyl fluoride (ABSF; 0.7 mM) and glycerol (100 ml $litre^{-1}$). Following centrifugation to remove cell debris, the supernatant was exchanged via Sephadex G25 into the same buffer but lacking ABSF. The assay method for EPSP synthase was essentially as described by Boerboom[3] except that [^{14}C]phosphoenolpyruvate (PEP) was at 0.5 mM, shikimate-3-phosphate at 0.75 mM, and 20 µl of plant extract was included in a final volume of 35 µl. After 3 min, assays were stopped by addition of ice-cold trichloroacetic acid (45 g $litre^{-1}$; 10 µl) and then rapidly neutralised prior to analysis by HPLC and radiometry. Extractable EPSP synthase activities of resistant [4.2 ($\pm$0.9) nmol EPSPS $min^{-1} mg^{-1}$ protein] and susceptible plants [3.65 ($\pm$0.9) nmol EPSPS $min^{-1} mg^{-1}$ protein] were indistinguishable. Also, there was no obvious trend in activity with growth stage (between two and five leaves). An apparent increase in extractable EPSP synthase activity of 20% was observed 16 h after application of 125 g ha^{-1} glyphosate to both resistant and susceptible biotypes. The conclusion that resistance was not based upon increased expression of EPSP synthase was further confirmed by comparative Northern blotting of the polyA mRNA fractions[4] from resistant and susceptible plants using a partial cDNA of the *Lolium* EPSP synthase gene as probe. If anything, the susceptible plants expressed higher levels of EPSP synthase mRNA. Southern analysis using the same probe indicated some similarities but also many differences between resistant and susceptible plants. These differences, which may have arisen partly from differences in methylation, underline the genetic diversity of this outcrossing weed.

EPSP synthase from resistant and susceptible plants were equally sensitive to inhibition by glyphosate [IC_{50} values of 1.4 ($\pm$0.25) and 1.2 ($\pm$0.25) mM, respectively under the above assay conditions].

For assays of DAHP synthase, plant extracts (as above) were first exchanged into K^+ Hepes buffer (10 mM; pH 7.3) containing DTT (1 mM), loaded onto a 1 ml MonoQ anion-exchange column equilibrated with the same buffer, washed with 0.1 M NaCl, and the active fraction eluted in 0.2 M NaCl. Assays were then carried out as described by Siehl.[2] Based on three extracts, the DAHP synthase activities of resistant plants were 7.5($\pm$2) nmol $min^{-1} mg^{-1}$ protein and from susceptibles, 5.6($\pm$2.2) nmol $min^{-1} mg^{-1}$ protein. It is not clear whether this slight difference is significant. On the basis of a single experiment, it appeared that pretreatment with glyphosate raised the extractable DAHP synthase activity in the susceptible plants to the same evel as that in the resistant.

Another possible basis for resistance would be reduced uptake or translocation of glyphosate. [^{14}C]Glyphosate was spotted onto a point just below the midpoint of the larger leaf of two-leaf-stage plants. At a number of timed intervals after treatment, the leaf was then washed to remove herbicide that had not been absorbed. Leaf and stem tissue at the site of application, above the site of application (the top of the leaf), and below the site of application (constituting the rest of the plant) was combusted and the [^{14}C]carbon dioxide released captured and counted. Not all of the original radioactivity was recovered and it is presumed that the missing 25–30% was in the roots or lost via the roots to the soil. Results, averaged between five replicates at each time point, were, nevertheless, very clear. Both resistant and susceptible plants rapidly absorbed the herbicide which, in both cases, was almost fully translocated with $<$4% remaining at the site of application 24 h after treatment, ($\sim$25% above and $\sim$30% below). Thus it seemed that glyphosate was readily absorbed and translocated by both resistant and susceptible plants.

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Preliminary investigations of glyphosate metabolism in resistant and susceptible plants have indicated that glyphosate is not rapidly metabolised by either biotype.

Application of glyphosate causes rapid accumulation of shikimate in treated plants and unregulated diversion of carbon may be the major reason for the herbicidal effect of glyphosate.[2] We therefore carried out an experiment to examine whether shikimate accumulates to any lesser extent in resistant plants. Plants were treated with 112.5 g ha^{-1} glyphosate and then, at intervals up to seven days, sets of 12 plants were pooled and analysed for shikimate.[5] Figure 1 shows that, following an initial similar rise, there was consistently lower accumulation of shikimate in treated resistant plants than in treated susceptible plants. Significantly, shikimate levels returned to pre-treatment concentrations after seven days in the resistant population, but not in the susceptible population. This would seem to be a very notable factor, underpinning the eventual survival of the resistant plants. Indeed the dynamics of the changes in shikimate concentration do seem to parallel the whole plant situation, where initial stunting is observed in the first few days, followed by survival and regrowth.

The above results presented a dilemma. There were no major differences in either the target pathway or in rates of uptake/translocation of glyphosate between the two biotypes. Nevertheless, on the basis of substrate accumulation, the degree of inhibition experienced by resistant plants was clearly reduced. Does less glyphosate reach the target site in resistant plants? The observation of significant cross-resistance (*c*4-fold, data not shown) to a structural analogue of glyphosate, 2-hydroxy-3-(1,2,4-triazol-1-yl)propyl phosphonate (TP) suggested that this may indeed be the case. TP is an experimental herbicide with a mode of action (inhibition of histidine biosynthesis) completely unrelated to that of glyphosate, but, like EPSP synthase, it also acts at a target site (imidazole glycerol phosphate dehydratase) located exclusively within the plastid.[6]

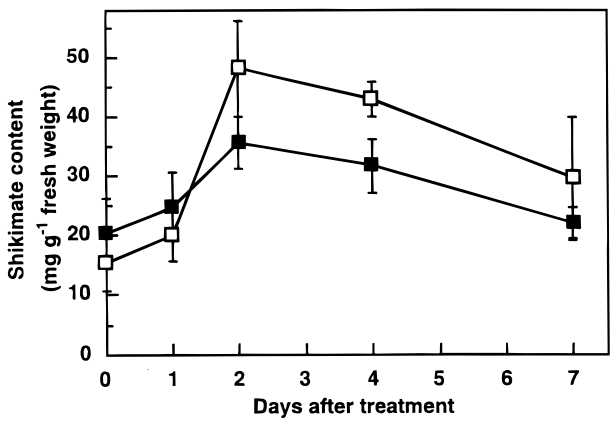

Figure 1. Shikimate accumulation in shoots of (□) susceptible and (■) glyphosate-resistant *Lolium rigidum* plants following the application of 112.5 g AE ha^{-1} glyphosate to two-leaf stage plants. Data are means (±S.E.) of six replications.

Significantly, there was no cross-resistance to more distantly related structures (eg phosphinothricin; see also Reference 1). It is thus tempting to speculate that at least one component of resistance (which may, of course, be multi-factorial) arises from reduced movement of glyphosate to its site of action in the plastid. Glyphosate and TP are structurally similar to phosphate esters shuttled between the plastid and cytoplasm. Movement of the herbicides may therefore be dependent upon the same molecular carriers as involved in the shuttles. Resistance to glyphosate based upon a mutation in such a carrier would be expected to be inherited as a recessive or partially dominant trait. Preliminary experiments indicate that this is indeed the case. We are currently looking at herbicide uptake into chloroplasts and examining the inheritance of resistance in more detail.

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Synthesis of analogues of the monic acids A and C as potential herbicides

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Abstract: The synthesis of some analogues of pseudomonic acids with simplified 'left-hand' side-chains is reported. Tested against isoleucyl tRNA, none was as active as methyl monate A and none was active as a herbicide in glasshouse tests.

Keywords: pseudomonic acid; monic acid; herbicide; herbicidal activity; isoleucyl tRNA synthetase

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